THE DISTRIBUTION OF GLYCOGEN AND LIPIDS IN HUMAN SKIN*

WILLIAM MONTAGNA, M.D.[1] HERMAN B. CHASE, M.D.[2] AND JAMES B. HAMILTON, M.D.[3]

INTRODUCTION

It is well known that human skin contains glycogen and lipids but the precise histological localization of these substances needs clarification. This study is based on specimens of human skin from the mons pubis, a region which abounds in coarse, growing pubic hairs, both eccrine and apocrine sweat glands and sebaceous glands. The following relatively new observations are emphasized: 1) the presence and distribution of glycogen in the epidermis, ducts of sebaceous glands, sebaceous glands, external root sheath of hair follicles, hair shaft and associated structures; 2) the relative abundance of glycogen and Schiff-reactive, diastase resistant substances in eccrine and apocrine sweat glands; 3) the distribution and partial characterization of lipids in epidermal cells and in sweat glands. Sebaceous lipids are not considered here.

MATERIAL AND METHODS

Surgical specimens from the mons pubis were obtained from 11 males, 10 white and one negro, 18 to 38 years of age. All of these men were mentally deficient but in good physcial condition. The excisions were performed under general anesthesia. After the skin had been shaved it appeared clear of any observable lesions. All of the specimens were collected from 9 to 10 A.M. Immediately after excision, strips of skin were fixed in Zenker-formol, for the study of glycogen and other substances. For the study of lipids tissues were fixed in formol-calcium and in 10% neutral formaldehyde.

In our experience, Zenker-formol has been a more satisfactory fixative for glycogen than the generally used picric-acid-alcohol-formol of Rossman. Glycogen and other polysaccharides were demonstrated on 5 μ paraffin sections treated with the periodic-acid Schiff technic of McManus (1). For the best results, the periodic acid was warmed to 37°C. and allowed to act on sections for 10 minutes rather than for the usual 5 minutes at room temperature. For control, alternate sections were immersed in buffered diastase for 45 minutes before treatment with the periodic-acid Schiff routine. Only Schiff-reactive material which is abolished by diastase will be called glycogen, the Schiff-reactive, diastase resistant materials represent other substances (Leblond, (2); Lillie, (3)).

In the study of lipids, formol-calcium fixed tissues were chromated after the procedure of Baker (4), then imbedded in gelatin and sectioned at 5 to 10 μ. Sections colored with Sudan black were used to study the distribution of lipids. Sample sections were treated with the Schultz test for unsaturated steroids. Untreated frozen sections were studied under pol-

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* From the Arnold Laboratory, Brown University, Providence, Rhode Island and State University Medical Center, State University of New York, Brooklyn, New York.

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arized light for birefringence. Sections of material fixed in formol-calcium but not post-chromed and not imbedded in gelatin, were mounted in glycerol and viewed under ultraviolet light for the study of autofluorescence in sudoriparous glands.

GLYCOGEN

Surface epidermis. Demonstrable glycogen is spotty in epidermal cells. The cells of the stratum basale are free of it but clusters of cells in the stratum spinosum, especially above the rete pegs, often show diffuse glycogen (fig. 1). It is most often found in the cells around the pilosebaceous exits, and in cells which surround small superficial corneal cysts. The melanin granules which are contained in the basal epidermal cells become faintly colored by the Schiff reagent but this color is not removed by treatment with diastase. The basement membrane is strongly Schiff-reactive but also diastase resistant.

Hair follicles. Nearly all of the components of the hair follicles are rich in glycogen. Beginning from the outside and proceeding inwardly, in the connective tissue sheath all of the fibroblasts contain glycogen granules (fig. 5). The basement membrane of the external sheath, however, is Schiff-reactive but diastase resistant. The external sheath throughout most of its thickness and length is virtually packed with glycogen (figs. 2–6). Glycogen is also abundant in the external sheath of other mammals as demonstrated by Johnson and Bevelander (5) and Bolliger and MacDonald (6). We know of no tissue which contains greater amounts of it. In the middle third of the follicle, the tall columnar basal cells of the external sheath contain so much glycogen that apparently little cytoplasm is left (fig. 2). The basal cells become low cuboidal in the upper part of the follicle, where the external sheath is continuous with the epidermis, and in the basal part of the follicle around the hair bulb. In these regions all of the cells of the outer sheath become smaller. Concomitant with this decrease in the size of the cells there is a progressive loss of glycogen. The internal sheath contains no glycogen throughout its length, not even near the hair bulbs (figs. 5, 6). The cuticle of the internal sheath is also devoid of glycogen. The cells of the cuticle of the cortex are rich in glycogen in the region just distal to the hair bulb and approximately for one third the length of the follicle or less (fig. 6). In the middle third and distally, where these cells are cornified, there is no glycogen. In the cortex of the hairs glycogen is found in the region immediately distal to the bulb and spottily throughout the lower one fourth or one third of the follicle. The medulla of the pubic hair is rich in glycogen in the region near the bulb (fig. 6). There is no stored glycogen in dermal papillae, but there are numerous Schiff-reactive diastase resistant fibers (figs. 3, 4).

Sebaceous glands. The cells of the ducts of sebaceous glands are packed with delicate glycogen granules, and many glycogen granules are found in all of the peripheral non-sebaceous sebaceous cells (figs. 11, 12). In cells which have begun to accumulate lipid droplets, as well as in moderately mature sebaceous cells, glycogen is still abundant, but it is absent from mature sebaceous cells and from the sebum.

Sudoriparous glands. Although Hoepke (7) and Bunting et al. (8) have described glycogen in eccrine glands, they do not give sufficient details concerning

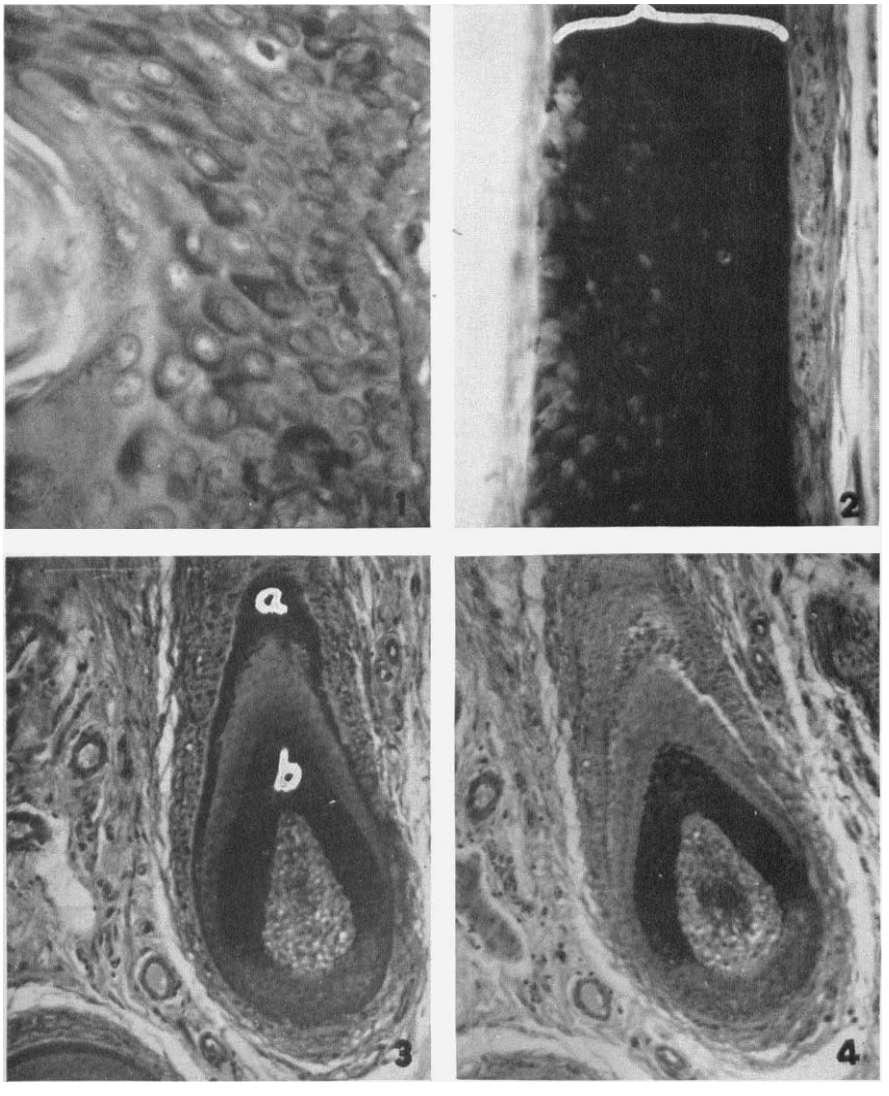


PLATE 1

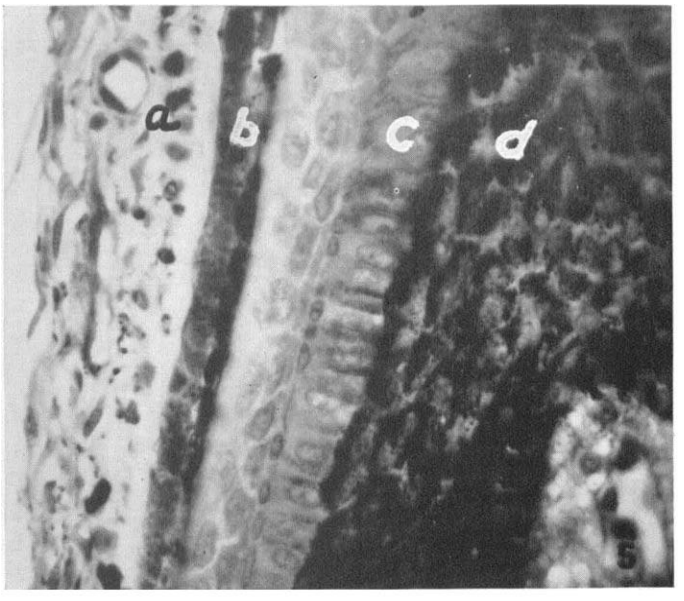
All of the tissues shown in these photomicrographs were fixed in Zenker-formol and treated with the periodic-Schiff reagent. The photographs have been taken through a dark green filter to sharpen the resolution of the red color. The nuclei were counterstained with hematoxylin.

FIG. 1. Glycogen in the stratum spinosum of the epidermis of a man 38 years old. Observe stratum basale on the right, with the dark pigment granules. Stratum granulosum on the left. Ca. 705×.

Fig. 2. Heavy glycogen deposit in external sheath in the skin of a man 19 years old. Ca. 470×.

FIG. 3. Tangential section through hair bulb to show glycogen in external sheath (a). The cone-shaped cortical mass (b) above the papilla is laden with pigment. From a man 22 years old. Ca. 125×.

FIG. 4. Section adjacent to the one in Fig. 3, digested with diastase. Compare with Fig. 3. Ca. 125×.



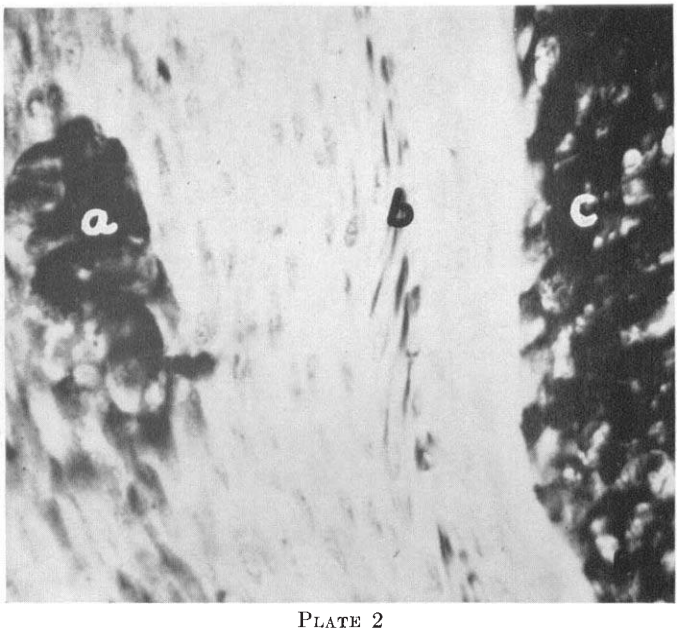


PLATE 2

FIG. 5. Enlarged detail of Fig. 3. Glycogen in the connective tissue sheath (a) and external sheath (b). Some glycogen in the culticle of the cortex (c). Cortex laden with melanin. Ca. 705×.

FIG. 6. Longitudinal section through follicle and lower part of hair shaft. Glycogen in external sheath (c) cuticle of cortex (b) and in medulla (a). From a man 19 years old. Ca. 705×.

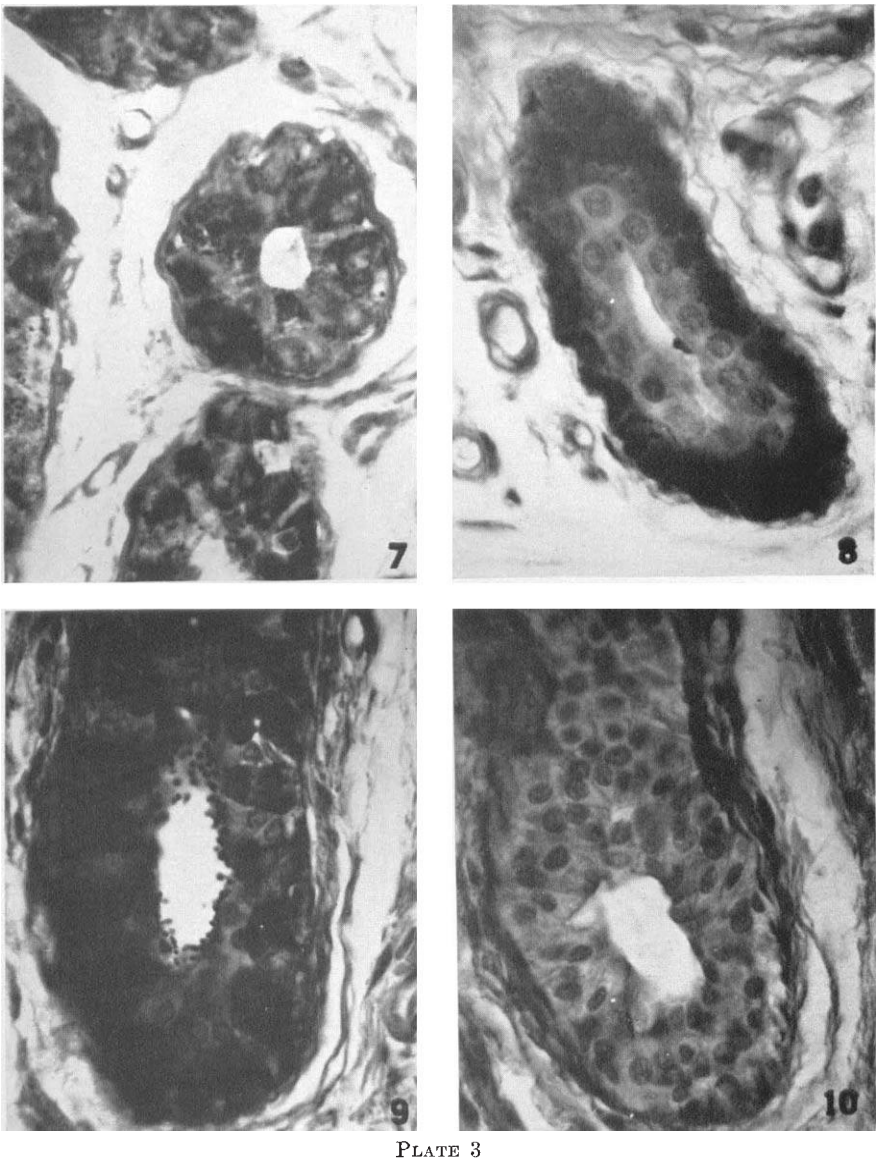


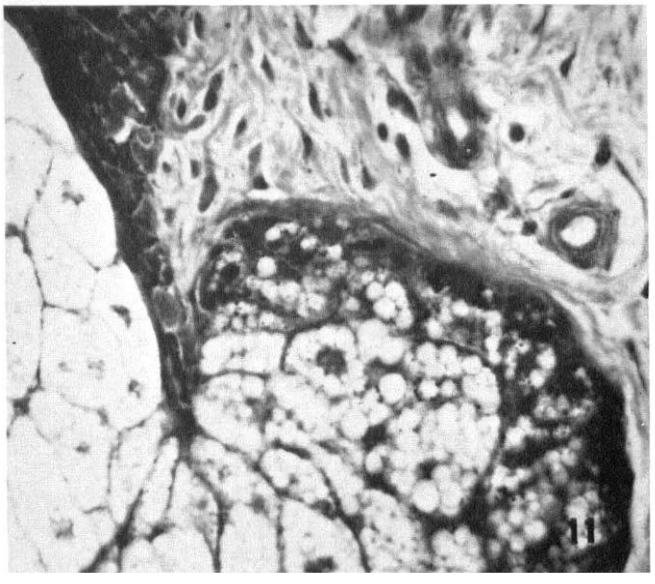
PLATE 3

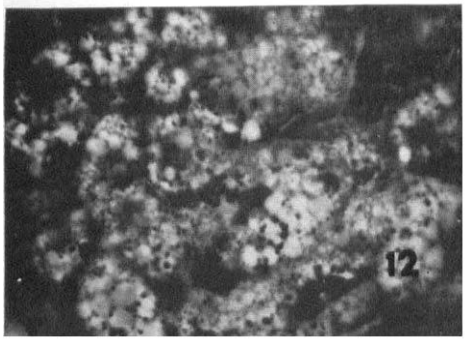
FIG. 7. Glycogen in secretory epithelium of eccrine gland. From a man 20 years old. Ca. 705×.

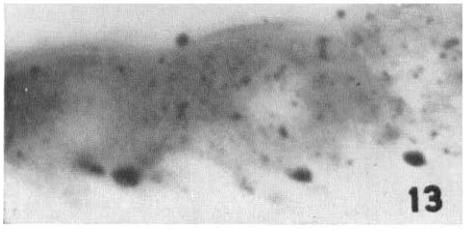
FIG. 8. Duct of apocrine gland. Observe glycogen in basal cells only. Same section as Fig. 7. Ca. 705×.

FIG. 9. Glycogen in apocrine gland. Same section as Fig. 7. Ca. 705×.

FIG. 10. Same tubule as in Fig. 9, in adjacent section, treated with diastase. There is some Schiff-reactive diastase resistant substance at the apex of the cells toward the lumen. Ca. 705×.







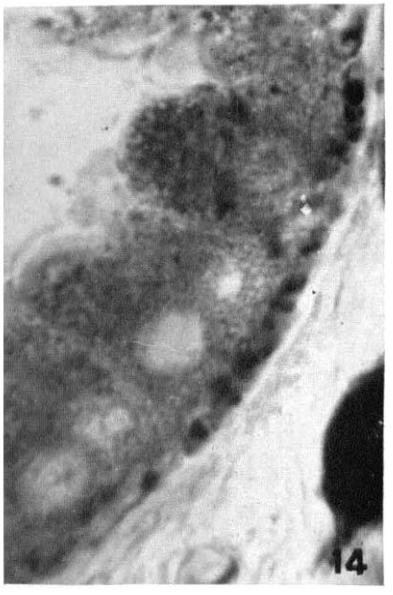


PLATE 4

FIG. 11. Glycogen in sebaceous gland. Observe abundant glycogen in duct at upper left, and at the periphery of sebaceous acinus. From a man 32 years old. Ca. 705×.

FIG. 12. As above. Glycogen abundant.

FIG. 13. Sudanophilic droplets in eccrine glands. Note especially the coarse droplets at the base of the cells; these are actually within the myoepithelial cells. From a man 21 years old. Ca. 1125×.

FIG. 14. Sudanophilic droplets in apocrine glands. Observe lipids in myoepithelial cells. Same section as above. Ca. 1125×.

the abundance and localization of this substance. Nearly all the cells of the secretory epithelium of eccrine glands contain glycogen. It is more abundant in some cells than in others (fig. 7). Comparison of diastase digested sections with sections not so digested reveals that the apices of the glycogen-rich cells contain masses of Schiff-reactive, diastase resistant granules. These granules correspond to the eosinophilic granules seen in preparations stained with Giemsa. The secretion in the lumen of these glands occasionally contains coarse glycogen granules, but more often shows abundant strongly Schiff-reactive diastase resistant substances. In the epithelium of the ducts of eccrine glands all of the cells, with the exception of those lining the lumen, have a stippling of glycogen in the cytoplasm (Bunting et al. (8), found none in the specimens they studied).

Apocrine glands are not numerous in the skin of the mons pubis, but nearly all sections contain a few. The secretory cells of these glands contain some glycogen granules at the base of the cells, and an abundance of Schiff-reactive diastase resistant material in the luminal cytoplasm, and in the lumen (figs. 9, 10). The myoepithelial cells contain only very fine glycogen granules. The epithelium lining the ducts is double layered; the basal cells contain glycogen granules (fig. 8) whereas the luminal cells do not.

LIPIDS

In this paper we shall omit a description of the lipids in sebaceous glands. These have been described previously in the sebaceous glands of the external auditory meatus by Montagna et al. (9) and they are now being investigated further by Doctor Raymond Suskind from the Kettering Institute in Cincinnati.

Epidermis and hair follicle. In the cells of the epidermis, external sheath and in the ducts of sebaceous glands there are small sudanophil bodies which are arranged either perinuclearly or at the poles of the nucleus. Most of these granules are very fine and are usually just within the resolving power of the microscope. Some of them, however, are coarser and contain a sudanophobic center. These coarser granules are often linked together to form short chains. The details of these elements have already been described by Montagna (20) in the epidermis of other mammals where these elements appear to correspond to the Golgi element or "liponchondria" of Baker (10). Perinuclear sudanophil bodies, then, are sharply defined in the epidermis, upper portion of the external sheath and in the ducts of sebaceous glands. In the lower two thirds of the external sheath, the cytoplasm of the cells is laden with glycogen and the lipid bodies are distorted, being often dispersed and ill-defined. The matrix cells of the bulb also possess perinuclear lipid bodies; in the upper bulb they are masked by pigment granules.

Sudoriparous glands. Bunting et al. (8) have given a good account of the lipids in sudoriparous glands. When treated with Sudan black, both eccrine and apocrine glands show large amounts of lipid in the secretory epithelium. There are variable amounts of coarse lipid globules in the apices of the cells (more numerous in apocrine glands), and the rest of the cytoplasm shows a delicate sudanophilic stippling. The myoepithelial cells, contrary to the statement of

Bunting et al. (8), contain coarse sudanophil droplets aligned on the long axis of the spindle cells (figs. 13, 14).

Under polarized light, we have never observed birefringent lipids in apocrine or eccrine glands. This is difficult to understand in view of the paper of Bunting et al. (8) in which they not only describe but show a clear photograph of abundant anisotropic lipids in an apocrine gland. In our material, even old specimens of skin maintained in the fixative for over one year, and in which all the adipose cells show rosettes of long birefringent crystals (fatty acids), sudoriparous glands show no anisotropic lipids.

Under near-ultraviolet light, the lipids in apocrine, and to a lesser extent, eccrine glands emit an orange-yellow autofluorescence, as described by Bommer (11), Hemperl (12), Popper (13), and Bunting et al. (8). According to Bunting, an acetone-soluble pigment, dissolved in the larger lipid droplets of the sudoriparous glands, is responsible for the autofluorescence.

The Schultz test reveals no green coloration indicative of cholesterol or esters of cholesterol. It must be assumed, then, that these substances are present in very small amounts, if at all.

DISCUSSION

Critical points in these observations are: a) the presence and distribution of glycogen in the epidermis, in the lower hair shaft, and especially in the external sheath; b) the relationship of glycogen to lipid storage in the sebaceous glands, and c) the abundance of glycogen, and its relation to Schiff-reactive, diastase resistant substances in sudoriparous glands. The demonstration of so much glycogen which has heretofore escaped observation is traceable to manipulations in technic. Comparison of adjacent sections, one of which has been treated with periodic acid at room temperature for 5 minutes and 30 minutes with the Schiff reagent, the other being treated for 10 minutes with periodic acid at 37° C and the Schiff reagent for 45 minutes, reveals much more glycogen in the second preparation than in the first. While the first method shows only traces of glycogen in sebaceous glands, the second shows an abundance of it. The only criterion for the identification of glycogen in tissue sections is a Schiff-reactive substance which is digested with saliva or diastase; therefore, the additional Schiff-reactive, diastase digestible substance revealed by the second method must be considered glycogen.

The amount and localization of glycogen in follicles and included pubic hairs are of considerable interest in relation to the development of the hair shaft. The hairs observed are in active phases of growth. In the actively dividing matrix cells of the bulb there is no glycogen, but immediately distal to the bulb, in the region of keratinization, there is glycogen in the medulla, a small amount in the cortex, and a considerable amount in the curved, spindle-shaped cuticle cells. At this same level there is no glycogen in the inner sheath but some in the external sheath. Further distally the glycogen in the cortex and cuticle disappears; the inner sheath has none in its entire length; the external sheath has tremendous amounts. Throughout the length of the follicle, the cells of the

external sheath are loaded with glycogen with the exception of the basal cells which have none in the upper and lower thirds of the follicle. Around the hair canal proper, distal to the opening of the sebaceous duct, glycogen diminishes in the external sheath as keratinization appears. The surface epidermis which is continuous with the upper portion of the external sheath, has no glycogen in the basal layer but in some places may have an appreciable amount in the spinous layer. The keratinized stratum granulosum and corneum contain no glycogen. The fibroblasts of the connective tissue sheath contain glycogen but the dermal papilla contains only Schiff-reactive diastase resistant material. It is apparent in these active follicles that glycogen is not found in the actively dividing cells of the matrix, and also not in the cells of the lower and upper basal layers of the external sheath. Glycogen, furthermore, is not found in the highly keratinized regions of the cortex, cuticle, and inner sheath. In the keratinization zone of the shaft, especially the lower portion, there is some glycogen present. Keratinization of cells of the medulla is slower and less complete than in the cortex or cuticle and glycogen extends further distally before disappearing. The failure of glycogen to appear in any part of the inner sheath may be associated with the extremely early and rapid keratinization of these cells after they have been formed from the basal matrix cells of the lower bulb (Chase et al., (14)). The absence of stored glycogen in the dermal papilla may be associated with the physiological activity of these cells. Glycogen is then found only in cells immediately following active proliferation, as in the whole middle third of the external sheath and inner layers of the upper and lower thirds, and the lower cells of the hair shaft. When cells are actively dividing, or are active trophically (dermal papilla), or when they are becoming keratinized, glycogen may be used up, rather than stored. The distribution of glycogen in other areas of human skin, and especially in areas where hair is in the resting phase, should be investigated in view of the observations made here.

Lombardo (15) and Sasakawa (16) describe glycogen in the epidermis, sebaceous glands and other cutaneous appendages of human fetuses younger than 6 months. In older fetuses and in the adult, glycogen becomes restricted to the external sheath and to sudoriparous glands. In psoriasis and other cutaneous irritations these authors find that glycogen reappears in the epidermis and sebaceous glands. Using the McManus routine on acetone-fixed material, Montagna et al. (9) described traces of glycogen in the sebaceous glands in the human external auditory meatus. In the present study, however, using better fixed material and lengthening the action of periodic acid, the sebaceous glands of the mons pubis reveal a considerable amount of glycogen. Whether or not glycogen is present also in the glands elsewhere in the body we do not yet know. Glycogen is found in the cells of the sebaceous ducts, in the peripheral non-sebaceous sebaceous cells and in the peripheral young sebaceous cells; none is found in the mature sebaceous cells. Since there is a gradual loss of glycogen with the increase in lipid storage, it appears that glycogen may represent an intermediary substance in the transformation of dietary carbohydrates to sebaceous lipids, a situation similar to that in adipose tissue. The presence of glycogen

in fat of animals fed a high carbohydrate diet has been known and it was investigated chemically by Tuerkischer and Wertheimer (21). The recent study of Fawcett (17) shows that in the normal rat, fat cells are ordinarily devoid of stainable glycogen; after the injection of a single large dose of insulin to normal animals and to animals being refed after fasting, glycogen is present in large amounts in both white and brown fat. The fat of diabetic animals contains no glycogen. Unlike white fat, the brown fat of normal mice shows cycles of accumulation and depletion of glycogen similar to that in the liver (Micklewright, 18). This similarity of the sebaceous glands to adipose tissue, and particularly to brown fat, makes possible the suggestion that sebaceous lipids may be synthesized by a local oxidative breakdown of glycogen.

Bunting et al. (8) find much glycogen in eccrine glands but only Schiff-reactive, saliva resistant substances in apocrine glands. On the contrary, we find large amounts of both substances in both types of glands, although glycogen is more abundant in eccrine glands. This discrepancy might be explained either by the greater sensitivity of the test as modified by us, or by a local difference between the glands of the axilla and those of the mons pubis. The apices of the cells of either eccrine or apocrine glands seldom contain glycogen, regardless of the amount in the body of the cell, but contain, instead, Schiff-reactive, diastase resistant granules. Similarly, although the lumen may contain glycogen (fig. 9) it more often contains a Schiff-reactive substance, which resists digestion with diastase.

The cells of the epidermis, external sheath, and hair bulb as well as those of the ducts of sebaceous glands and the peripheral, non-sebaceous sebaceous cells possess perinuclear sudanophil granules which are similar to the argyrophilic dictyosome of Parat (19) and thus similar to the Golgi element as described in the epidermis of other mammals by Montagna (20).

Our observations on the distribution and characterization of lipids in sudoriparous glands, with two exceptions, are in agreement with those of Bunting et al. (8), the exceptions being that we find, a) coarse lipid droplets in myoepithelial cells and b) the lipids in sudoriparous cells are never anisotropic.

SUMMARY

In the epidermis, glycogen is demonstrated in groups of cells in the stratum spinosum, especially above rete pegs and around the orifices of pilosebaceous units. In hair follicles, glycogen is found in the fibroblasts of the connective tissue sheath; it is most abundant in the cells of the external sheath; there is some in the cuticle of the cortex, in the cortex and in the medulla just above the hair bulb. There is no glycogen in the internal sheath, matrix cells and dermal papilla.

Both eccrine and apocrine glands contain abundant glycogen and Schiff-reactive, diastase resistant substances. Glycogen is usually at the base of the cells, the other substances at the apex. Since glycogen is seldom secreted into the lumen, it is likely that it is an intermediary product.

In sebaceous glands, glycogen and lipids are present together in the peripheral

cells, and the amount of glycogen is inversely proportional to the amount of lipids stored. Mature sebaceous cells contain no glycogen.

The cells of the epidermis, external sheath, duct of sebaceous glands, and those of the hair bulb contain delicate, perinuclear sudanophil granules which are comparable to Golgi bodies.

Sweat glands, both eccrine and apocrine, contain autofluorescent, isotropic, Schultz-negative lipids. The myoepithelial cells contain coarse lipid droplets.

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